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Are intra-pleural bacterial products associated with longer survival in adults with malignant pleural effusions? A systematic review

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ABSTRACT

Background: Intra-pleural bacteria are effective pleurodesis agents in malignant pleural effusions. However, their relationship with survival is unclear.
Objectives: We undertook a comprehensive, structured evaluation of survival outcomes in adults with malignant pleural effusions treated with intra-pleural bacterial products.
Data sources: Medline, Embase, Cochrane library, Clinical Trials Registers and Open Grey.
Study eligibility criteria, participants, and interventions: Randomised controlled trials and non-randomised comparative studies were included, if the population included adults with malignant pleural effusions. Interventions of interest were any intra-pleural bacterial product, compared with placebo, alternative intra-pleural drug, or no treatment. Survival outcomes were collected.

Study appraisal and synthesis methods: Two reviewers independently screened studies for eligibility, assessed papers for risk of bias and extracted data. Narrative synthesis was performed as high heterogeneity between studies precluded meta-analysis.
Results: 631 studies were identified, of which 14 were included. All were at high or unclear risk of bias in at least one domain. Six studies reported a survival benefit associated with intra-pleural bacterial products, whilst 8 reported no difference. Non-randomised studies and studies published prior to 2000 were more likely to report survival benefits.
Limitations: There was high heterogeneity between studies, which limited the generalisability of findings. Publication bias may have affected the review as five full-text papers were unobtainable, and survival outcomes were missing in a further five.
Conclusions: There is a lack of high quality evidence regarding the relationship between intra-pleural bacterial products and survival.

Implications of key findings: Well-designed, prospective randomised trials are needed, to determine whether intra-pleural bacterial products can improve survival in pleural malignancy.
PROSPERO registration number: CRD42017058067.

1. Background

Malignant pleural effusions (MPE) arise as a result of primary pleural tumours, i.e. malignant pleural mesothelioma (MPM), or metastatic spread from distal tumours, most commonly lung cancer [1]. The presence of MPE usually reflects advanced or metastatic disease, and consequently treatment is primarily palliative, with fluid management a priority [2–4].

Administering an inflammatory agent into the pleural space to achieve pleurodesis is an effective way of controlling fluid and improving breathlessness, but has no effect on the underlying disease process. [1,3,5,6], Historically, pleurodesis was undertaken using bacterial products such as *Corynebacterium*] parvum, and in certain countries these products are still used [7–10]. Some clinicians believed these products exerted an anti-tumour effect alongside their pleurodesis properties [11–13]. The hypothesis was based on evidence that MPE were associated with local immune inhibition, and that survival correlated with the ability to maintain intra-pleural immune activity [14–19]. Bacterial products were recognised as potent stimulators of the immune response, and hence an early theory of immunotherapy was developed. This was supported by observational studies that suggested pleural infection was associated with longer survival following

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surgery for lung cancer [20–22].

The concept evolved through the 1970 s with clinical trials evaluating the role of intra-pleural BCG after lung cancer surgery. Trial data were conflicting, and the practice was not adopted into routine care [23–27]. However, BCG found a role as an intra-vesical treatment for bladder cancer, suggesting some anti-neoplastic activity [28].

Recently, interest in immunotherapy has resurfaced, and several systemic immunotherapy products have been adopted into routine use for other cancer types [29–35]. Interest in intra-pleural bacterial products has also risen, with agents such as *Staphylococcus* superantigen, *Lactobacillus* casei and streptococcal preparations undergoing investigations in clinical studies [36–38].

To date, the literature on intra-pleural bacterial products and their relationship with survival has not been systematically reviewed. We aimed to undertake a comprehensive evaluation of the evidence, with meta-analysis of RCT data if possible, to answer the question "Are intra-pleural bacterial products associated with longer survival in adults with MPE?"

2. Methods

2.1. Registration

The review was registered on PROSPERO International Prospective Register of Systematic Reviews, registration CRD42017058067. A summary of the protocol is available at https://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017058067.

2.2. Data sources

An electronic literature search was undertaken using MEDLINE (1946 to Present), EMBASE (1974–2017 week 09), Cochrane Database of Systematic Reviews, Cochrane CENTRAL Register of Controlled Trials, International Clinical Trials Registry (ISRCTN), EU Clinical Trials Register, US NIH Clinical Trials Register and Open Grey (System for Information on Grey Literature in Europe – SIGLE).

Once the initial electronic search was complete, a manual search was undertaken to review the references of included papers and systematic reviews, to ensure all relevant papers were captured.

2.3. Search strategy

The search strategy for each database is shown in Appendix A. The strategy included exploded MeSH headings for MPE, combined with keyword or title word searches for intra-pleural bacteria, immunotherapy and specific products. The initial search was performed on 28/02/17 and was repeated on 22/02/18 to identify studies published in the intervening year.

2.4. Eligibility criteria

2.4.1. Types of study

RCTs were included, as were non-randomised comparative studies. Non-randomised studies included case-control studies, comparative cohort studies and matched case series, prospective or retrospective. Studies with no comparison group were excluded, as were informal review articles, editorials, conference abstracts, animal or in vitro studies and studies where no abstract was available. Systematic reviews were included and used to identify potentially eligible studies not identified by the search.

Clinical trials registers were searched. If the timelines suggested the trial had been completed but not reported, the authors were contacted and asked to provide the data.

Research papers in all languages were included. Foreign language papers were translated into English using an online translation service. No date limitations were placed on the search.

2.4.2. Types of participants

Studies were eligible if they included adults with MPE due to any underlying tumour. Studies were excluded if they included a mixed population of benign and malignant effusions, unless there was a clear distinction in reporting the results for the two groups. Similarly, studies that included participants with other effusions (e.g. ascites) were excluded unless the results were reported separately for each effusion type. Studies that included a surgical cohort were excluded as pleural involvement is usually a contra-indication for cancer surgery.

2.4.3. Types of interventions

The intervention was intra-pleural delivery of any bacterial product including, but not limited to, *Corynebacterium* parvum, BCG, *Staphylococcus* superantigen, *Lactobacillus* casei, OK432 and lipopolysaccharides. Studies in which bacterial preparations were delivered via other methods were excluded. Studies assessing viral vectors, vaccine therapy, fungal extracts or synthetic immunotherapies were excluded.

2.4.4. Types of comparators

Comparators included no treatment, placebo or alternative non-bacterial intra-pleural product.

2.4.5. Types of outcomes

The outcome of interest was survival. Outcomes relating to pleural effusion size, pleural effusion control or pleurodesis were not collected as this data has been reviewed in a recent Cochrane meta-analysis [6]. If an article referred to unpublished data that may have met the eligibility criteria, the authors were contacted and asked to provide raw data.

2.4.6. Screening & study selection

The titles and abstracts of studies identified by the search were screened for eligibility and potential studies obtained in full-text format and reviewed.

2.4.7. Assessment of risk of bias

Included studies were assessed using the Cochrane risk of bias tool [39].

2.4.8. Data extraction

Data were extracted from included studies using the form shown in Appendix B. If a study stated in its methodology that data relevant to the PICO criteria was collected, but did not report this data, the authors were contacted and asked to provide the data.

Abstract screening, full-text review, risk of bias assessment and data extraction were undertaken by two reviewers, independently. Discrepancies were resolved by discussion, or by consultation with a third party.

2.4.9. Data analysis

Odds ratios were calculated with 95% confidence intervals (95% CI) for proportional outcomes, where possible. Hazard ratios (and 95% CI) were extracted for time to event data, or calculated using Cox Proportional Hazards Model if sufficient data were available. Where comparative statistics could not be calculated, simple descriptors were reported with measures of variance as reported in the original studies.

Meta-analysis was planned if two or more RCTs were identified with low risk of bias in the randomisation domain, provided the data were comparable. Heterogeneity was expected to be high, therefore a random effects model was planned. Heterogeneity would be assessed visually with Forest plots, and using the I2 statistic [40]. Where insufficient data were available for meta-analysis, and for studies with a high risk of bias, narrative synthesis was performed.

Univariable meta-regression and Fishers exact test for heterogeneity were used to explore the relationship between study design, year of publication, patient population and bacterial product studied and the

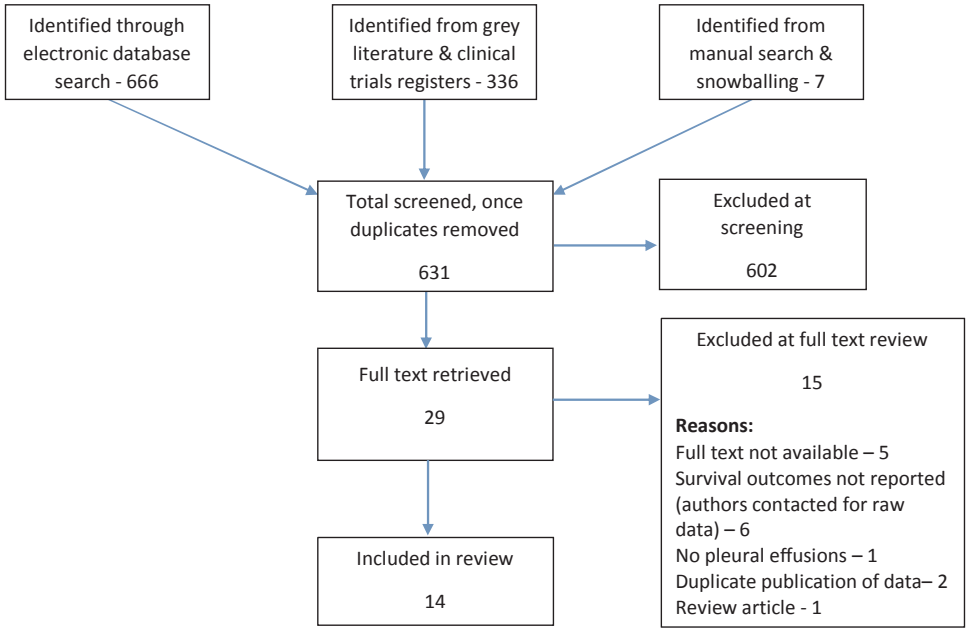

Fig. 1. PRISMA diagram for search and subsequent review.

likelihood of the study reporting a positive effect.

3. Results

3.1. Literature search & eligibility

631 articles were identified by the search, once duplicates were removed. Of these, 602 were excluded at screening, and 29 full-text manuscripts were retrieved. 15 [41]articles were excluded at full-text review: full-text was unavailable for 5; survival outcomes were not reported, or not reported separately, in 6; 2 publications reported duplicate data (in both cases the paper with the least amount of data was excluded); 1 paper had no data on pleural effusions and 1 paper was a review article. Thus 14 articles were included. The PRISMA diagram is shown in Fig. 1. A list of papers excluded at full-text stage is available in Appendix C, with reasons for exclusion.

3.2. Characteristics of included studies

Of 14 papers included, 8 were RCTs [7,37,38,42–46], and 6 were non-randomised comparison studies, most commonly cohort studies with historic comparators [13,36,41,47–49]. Studies were published between 1979 and 2007, with 6 undertaken during or after 2000. [36,41–43,46,47],Study characteristics of RCTs and non-randomised studies are shown in Table 1 and Table 2 respectively.

The most frequent population was patients with MPE due to lung cancer (all sub-types) [37,38,45,48,49], or non-small cell lung cancer (NSCLC) [36,41–4346]. Other studies included patients with MPE due to any underlying tumour [7,13], MPE due to MPM [47] and MPE secondary to lung and gastrointestinal tumours [44].

Several bacterial products were used, including OK432 in 6 studies, [38,41,42,44,46], *Corynebacterium* parvum in 3, [7,13,47], BCG cell wall skeleton in 2, [48,49], and *Lactobacillus* casei [37], *Staphylococcus* aureus superantigen [36], and *Nocardia* rubra cell wall skeleton [45] in 1 apiece. Comparators included intra-pleural chemotherapy, [7,13,37,38,41,42,44–46], usual treatment, [48,49], talc poudrage [36], or alternative pleurodesis agents [47]. One RCT compared two doses of the same bacterial product [43]. Two studies employed a three-arm design, comprising bacterial product alone, chemotherapy alone, and bacteria/chemotherapy combination. [42,44]

3.3. Risk of bias assessment

Risk of bias was high or unclear in at least one domain for all studies, as shown in Table 3.

Half the included RCTs (4/8) were published prior to the development of the Consolidated Standards of Reporting Trials (CONSORT) guidance, and consequently much of the information required to assess risk of bias was lacking. Information on random sequence allocation and concealment of allocation was provided in only 3 trials. [37,38,44], Information on blinding of participants and outcome assessments was lacking in all but two RCTs. [37,45], Selective reporting and incomplete outcome data were commonplace, with only three RCTs including all participants in the final analysis and reporting all outcomes stated in the methodology. [42,43,46],

All non-randomised studies were at high risk of selection bias, as well as high risk of performance and ascertainment bias, since none were blinded. Although the latter two biases are less pertinent in the context of an objective outcome measure such as survival, the lack of blinding enabled selective reporting of outcomes, which introduced further potential bias. All 6 non-randomised studies were at risk of other biases or confounding, as described in Section 3.6.

3.4. Synthesis of results

Six of the fourteen included studies reported a survival benefit associated with intra-pleural bacterial immunotherapy. [13,36,37,45,48,49], This ranged from 2.5 months to 5.4 months from the date of drug administration. However, no confidence intervals were reported so it was impossible to evaluate the precision of these estimates. The remaining eight studies demonstrated no difference in survival between patients treated with intra-pleural bacteria and comparators. [7,38,41–44,46,47],

Only one paper provided a measure of variance for the survival estimate, specifically 95% CI [36]. One paper provided patient-level data, and survival analysis was undertaken by one of the reviewers (ACB) [49]. No other measures of variance were available and requests for raw data were unsuccessful, therefore meta-analysis was not possible. Additionally, high heterogeneity within and between populations, interventions and comparators meant meta-analysis was inappropriate, even using a random-effects model. Consequently, narrative synthesis

Table 1
Summary of randomised trials included in the review [7,37,38,42–46].

1st author	Publication date	N	Study popn	Intervention (& dose)	Comparator (& dose)	MST intervention, days (95% CI)	MST comparator, days (95% CI)	P value
Ishida[42]	2006	49	MPE 2° to NSCLC	OK423 (5KE)	Cisplatin alone (50mg)	131	152	p=0.55
				OK432 (5KE) + cisplatin (50mg)		256		
Kasahara[43]	2006	40	MPE 2° to NSCLC	OK432 (10KE)	OK432 (1KE)	235	158	Not reported
Luh[38]	1992	55	MPE 2° to lung cancer	OK432 (10KE)	Mitomycin C (8mg)	177	156	NS
Yoshida[46]	2007	105	MPE 2° to NSCLC	OK432 (0.2KE/kg)	Bleomycin (1mg/kg)	337 (186.9–408.8)	225 (151.2-265.3)	NS
					Etoposide (80mg/m²) + cisplatin (80mg/m2)		320 (240.8-399.7)	
Nio[44]	1999	42	MPE 2° to lung or GI cancer	OK432 (1-10KE)	i/v and i/p chemotherapy (various agents & doses)	51	74	p=0.530
				OK432 (1-10KE) + i/p chemotherapy (various agents & doses)		115		p=0.080
Masuno[37]	1991	95	MPE 2° to lung cancer	Lactobacillus casei (0.2mg) + doxorubicin (40mg)	Doxorubicin alone (40mg)	232	125	p=0.0061
Millar[7]	1980	21	Any MPE	C. Parvum (7mg)	Mustine (20mg)	80 (mean)	86 (mean)	NS
Yamamura[45]	1983	68	MPE 2° to lung cancer	Nocardia rubra (400mcg) + doxorubicin (40mg)	Doxorubicin alone (40mg)	266	190	p<0.05

Abbreviations: 95% CI – 95% confidence intervals, C. Parvum – Corynebacterium parvum, GI – gastrointestinal, HR – hazard ratio, i/p – intra-pleural, i/v – intravenous, KE – Klinische Einheit, kg – kilogram, mcg – microgram, mg – milligram, MST – median survival time, MPE – malignant pleural effusion, NS – non-significant, NSCLC – non-small cell lung cancer.

was undertaken.

3.5. Results of individual studies – randomised trials

Five RCTs investigated OK432, a heat- and penicillin-killed *Streptococcus* pyogenes preparation. An initial dose-finding trial found that 10 Klinische Einheit (KE) of OK432 was associated with longer survival than a dose of 1KE (33.6 weeks vs 22.6 weeks) but interpretation of these results is difficult without a non-OK432 comparator group [43].

Other trials compared OK432 with intra-pleural chemotherapy. The most methodologically robust was a three-armed study that compared OK432 at a dose of 0.2KE/kg with two different intra-pleural chemotherapy regimens in MPE secondary to NSCLC [46]. No survival difference was seen across the three groups. Similarly, Luh et al found no difference in survival between lung cancer patients treated with intra-pleural mitomycin C or OK432 (10KE) [38]. This paper was at risk of selective reporting, however, as outcomes were not stated a priori, and some participants were excluded from the analysis due to early death.

Ishida et al reported a trend towards longer survival in patients with MPE secondary to NSCLC treated with 5KE of OK432 and intra-pleural cisplatin, compared with OK432 alone or cisplatin alone [42]. Median survival was 8.3 months in the combination arm, compared with 5 months for cisplatin and 4.3 months for OK432 alone. Statistical significance was not achieved ($p = 0.55$), however, the trial was underpowered as only 49 patients participated – a sample size that was based on time constraints rather than formally calculated.

The final study to evaluate OK432 used different combinations of intra-venous and intra-pleural chemotherapy compared with varying doses of OK432, alone or in combination with intra-pleural chemotherapy [44]. The heterogeneity of regimens makes the results difficult to interpret, and the use of an comparator with a proven survival benefit (intravenous chemotherapy) in one arm must be taken into account when considering the results. No difference in survival was seen between the chemotherapy and OK432 arms, however the combination of OK432 and intra-pleural chemotherapy appeared to be associated with longer survival compared with OK432 alone (115 days vs 51 days). Given that intra-venous chemotherapy was given to a proportion of the control arm, this result could be interpreted to mean intra-pleural OK432 is as effective as standard of care chemotherapy. However, the lack of detail provided in the results and the fact the study

Table 2
Summary of non-randomised studies included in the review [13,36,41,47–49].

1st author	Publication date	Study design	Study popn	N	Intervention (& dose)	Comparator (& dose)	MST intervention, months (95% CI)	MST comparator, months (95% CI)	Significance
Ren[36]	2004	Case series with historic controls	MPE 2° to NSCLC	32	Superantigen of Staph aureus (100-400 mcg)	Talc	7.9 (5.9-11.4)	2.5 (1.3-3.4)	p=0.044
McLeod[13]	1985	Cohort with historic controls	Any MPE	67	C. Parvum (7mg)	Mustine (20mg)	8.2 (mean)	3.9 (mean)	p<0.01
Senyigit[47]	2000	Case series	MPE 2° to MPM	138	C Parvum (7mg)	Oxytetracycline (35mg/kg)	10	11	NS
						Nitrogen mustard (0.4mg/kg)		9	
Shimizu[41]	2005	Retrospective case control	MPE 2° to NSCLC	32	OK432 (dose not stated)	Cisplatin (80mg/m2)	14	18	NS
Yamamura[48]	1979	Cohort with historic controls	MPE 2° to lung cancer	87	BCG cell wall skeleton (200-400mcg)	Usual treatment	~10	~6	p=0.016
Yasumoto[49]	1979	Cohort with historic controls	MPE 2° to lung cancer	30	BCG cell wall skeleton (5mg) + chemotherapy	Usual treatment	8* (6.2-18.2)	4* (3.9-7.0)	p=0.016*

Abbreviations: 95% CI – 95% confidence intervals, C. Parvum – Corynebacterium parvum, GI – gastrointestinal, HR – hazard ratio, I/P – intra-pleural, I/V – intravenous, KE – Klinische Einheit, kg – kilogram, mcg – microgram, mg – milligram, MST – median survival time, MPE – malignant pleural effusion, NS – non-significant, NSCLC – non-small cell lung cancer.
*Survival analysis undertaken by reviewer (ACB).

Table 3
Table demonstrating risk of bias of included papers [7,13,36–38,41–44,47–50].

		Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other
Randomised trials	Ishida et al[41]							
	Kasahara et al[42]							
	Luh et al[38]							
	Yoshida et al [45]							
	Nio et al[43]							
	Masuno et al[37]							
	Millar et al[7]							
	Yamamura et al (1983)[44]							
Non-randomised studies	Ren et al[36]							
	McLeod et al[13]							
	Senyigit et al[47]							
	Shimizu et al[48]							
	Yamamura et al (1979)[49]							
	Yasumoto et al [50]							

Red – high risk of bias, green – low risk of bias, yellow – unclear risk of bias.

was not a formal non-inferiority design, make this likely to be an over-interpretation.

Yamamura et al also combined intra-pleural chemotherapy with a bacterial product, using Nocardia rubra cell wall skeleton in conjunction with doxorubicin, or doxorubicin alone in 68 participants with MPE secondary to lung cancer [45]. The nocardia group had a median survival of 266 days, compared with 190 days for single-agent doxorubicin ($p < 0.05$). However, patients who died within 30 days of randomisation were excluded from the analysis, risking attrition bias. Furthermore this outcome was the result of sub-group analysis of data from a larger trial in which multiple analyses were undertaken, without pre-specification in the analysis plan. Consequently reporting bias is possible.

Another RCT randomised 95 participants with lung cancer MPE to receive intra-pleural *Lactobacillus* casei and doxorubicin or doxorubicin alone [37]. Patients who received *Lactobacillus* had a median survival of 232 days compared with 125 days in controls ($p = 0.0061$). However, 19 patients were excluded from the final analysis, generating a high risk of attrition bias. Interestingly, no further trials were undertaken using either *Lactobacillus* casei or *Nocardia* rubra despite these seemingly positive results.

The final RCT investigated *Corynebacterium* parvum by randomising 21 participants to either *Corynebacterium* or intra-pleural mustine [7]. No survival difference was seen in this small study, with mean survival of 80 days for *Corynebacterium* and 86 for mustine. No information was provided about the distribution of the data, so it is unclear whether use of mean survival values was appropriate. Additionally, outcomes were not specified a priori, generating potential reporting bias.

3.6. Results of individual studies – non-randomised studies

Two non-randomised studies evaluated BCG cell wall skeleton (BCG-cws) alongside standard care in patients with lung cancer. [48,49], The first reported median survival of 10 months in 55 patients treated with BCG-cws, compared with 6 months in 32 age-matched historic controls ($p = 0.016$) [48]. The second presented patient-level data for 13 patients with MPE given BCG-cws and 17 historic controls [49]. These data were analysed by the reviewers to reveal median

survival of 8 months in the BCG-cws group and 4 months in controls, with a hazard ratio of 0.374 (95% CI 0.168-0.833, p = 0.016). However, both studies were at risk of selection bias, and the latter included a greater proportion of women in the BCG-cws group, a factor known to be associated with longer survival in lung cancer [50]. Additionally the use of historic controls may have introduced confounding due to advances in care happening between the two periods.

Two observational studies investigated *Corynebacterium* parvum. McLeod et al retrospectively analysed data from 67 patients with MPE treated with *Corynebacterium* parvum or intra-pleural mustine at a single UK centre [13]. Mean survival was 251 days in the *Corynebacterium* group compared with 119 days in the mustine group ($p < 0.01$). However, 14 patients died within 30 days of treatment and were excluded from the final analysis, introducing attrition bias. Additionally, patients who received mustine were treated prior to 1980, whilst the majority of *Corynebacterium* patients were treated after this, raising the possibility of temporal confounding. Furthermore, 6 patients who failed pleurodesis with mustine were excluded from the analysis, generating reporting bias.

Senyigit et al also investigated *Corynebacterium* parvum, but in patients with MPM [47]. They described 117 patients who received intra-pleural *Corynebacterium*, oxytetracycline or nitrogen mustard. No survival difference was detected between the three groups, with mean survival of 10 months in *Corynebacterium* patients, 11 months in oxytetracycline patients and 9 months in patients treated with nitrogen mustard. The study was at risk of attrition bias as 27 participants were excluded from the final analysis due to death, disease progression or loss to follow up. In addition, time-to-event modelling was not employed for survival analysis and thus censored data was not taken into account.

Shimizu and colleagues evaluated 32 patients with NSCLC treated with either intra-pleural OK432 or cisplatin between 2000 and 2004 [41]. They found no difference in survival, with median survival of 14 weeks in OK432 patients and 18 weeks in the cisplatin cohort. However, the two groups were markedly dissimilar, with worse prognostic characteristics in the OK432 group and less systemic chemotherapy administered in this group too. These differences could have attenuated a potential survival benefit associated with OK432, although the lack of observed survival difference is consistent with previous RCT data. [38,42–44,46],

Finally, Ren and colleagues gave intra-pleural staphylococcus superantigen (SSAg) to 14 patients with MPE secondary to NSCLC, six of whom also received intra-venous SSAg. Outcomes were compared with 18 historic controls from up to 10 years earlier. Median survival was 7.9 months in SSAg patients, compared with 2 months in controls (p = 0.0023), leading the authors to conclude that SSAg had anti-neoplastic effects. However, the study was vulnerable to a number of confounding factors, mainly relating to the differences between the two treatment groups. As well as the temporal divide between cases and controls, controls were treated in the USA whilst cases were recruited and treated in Japan. Differences in epidemiology, tumour and population genetics, healthcare systems, and treatment approaches between the 2 countries make interpretation difficult.

3.7. Meta-regression

A greater proportion of non-randomised studies reported favourable survival in the intervention group compared with RCTs (4/6; 66.7% vs 2/8; 25%) although this result was not statistically significant (p = 0.227). More studies undertaken prior to 2000 were positive compared with studies published after that date (4/8; 50% vs 1/6; 16.7%), and meta-regression supported this trend (odds ratio 0.88, 95% CI 0.77–1.00, p = 0.051)). A greater proportion of studies involving lung cancer patients were positive (4/6; 66.7%), compared to MPE secondary to NSCLC (1/5; 20%), any tumour (1/2; 50%) or MPM (0/1; 0%), but this difference was not significant (p = 0.467).

No specific bacterial product appeared more effective, although RCTs of *Lactobacillus* and *Nocardia* rubra were both positive, and BCG cell wall skeleton yielded positive results in both non-randomised studies that utilised it. [37,45,48,49], OK432 was not associated with any survival benefit in 5 randomised and 1 non-randomised studies, [38,41–44,46], although for one of those studies the comparator was intra-venous chemotherapy, suggesting that OK432 may be as effective as standard treatment [44]. *Corynebacterium* parvum was associated with longer survival in one non-randomised study [13], but no effect in 2 others (1 randomised7 and 1 non-randomised58), whilst *Staphylococcus* aureus superantigen was associated with longer survival in a single observational study [36].

4. Discussion

This systematic review evaluating the effect of intra-pleural bacterial products on survival in malignant pleural effusions is the first to formally summarise the literature for this topic. The review revealed a lack of high quality evidence, with all 14 studies suffering from high or unclear risk of bias in at least one domain.

The methodologies and results of the included studies were highly heterogeneous and consequently meta-analysis was not possible. Six studies reported a survival benefit associated with intra-pleural bacterial products, whilst eight found no difference. No specific agent appeared more likely to be associated with a survival benefit and no particular underlying disease was more likely to respond to intra-pleural bacteria. A higher proportion of non-randomised studies reported positive results, suggesting selection bias may have affected these results.

4.1. Implications for practice & future research

The current literature does not support the use of intra-pleural bacterial products as anti-neoplastic treatment in MPE.

There are several possible interpretations for the findings of this review. Firstly, there may be no relationship between intra-pleural bacterial products and survival. Alternatively, a relationship may exist, but existing studies have failed to demonstrate it. Finally, the variety of agents, doses and administration regimens used in the different studies may have

obscured a genuine effect related to a single agent or specific dose.

With respect to the latter point, it is accepted that different bacterial species and strains elicit differing degrees of immunological responses. For example, gram positive and gram negative bacteria induce different patterns of cytokine release with varying, and sometimes opposing, down-stream cellular responses [51,52]. Similarly different bacterial strains or preparations can have widely varying effects. Therefore it is plausible that the lack of consistent effect for any single agent could be a result of some studies, for example those with positive outcomes, using one strain whilst negative studies used an alternative, less immunogenic strain or preparation. Additionally, doses and administration regimens varied, with optimal dosage unknown for all the bacterial products studied. Apart from one randomised trial of OK432 [43], no formal dose-finding studies have been published for any of the bacterial agents. This could have resulted in the use of sub-therapeutic doses with consequent apparent inefficacy.

An alternative explanation is that the studies failed to detect an existing effect. Small sample sizes and the fact that survival tended to be a secondary outcome measure meant that the majority of studies were under-powered to detect differences in survival. In fact, several of studies were pilot projects with no formal sample size calculation undertaken. In the absence of meta-analysis, negative results from small individual studies should be interpreted with caution.

Finally, it may be the case that bacterial products have no effect on survival, despite good evidence that they are effective pleurodesis agents [6]. A similar effect was seen when chemotherapy agents were

administered intra-pleurally in malignant effusions – the drugs effectively caused pleurodesis but did not affect the underlying cancer or alter survival. [53–56], A possible explanation for this is that drugs administered into the pleural cavity have limited absorption into the systemic circulation. This has been shown to be the case with intra-pleural fibrinolytics in empyema. [57–59], Since most MPE arise as a result of metastatic disease, with at least one tumour located anatomically distant from the pleura, the lack of systemic absorption following intra-pleural administration limits exposure of distal tumours to the agent, hence efficacy is reduced.

By this theory, intra-pleural drug administration could be an effective approach for localised tumours i.e. malignant pleural mesothelioma (MPM). Delivering the drug directly into the pleural space would result in high concentrations of the therapeutic agent in the precise area where its activity is required, whilst simultaneously reducing the risk of side effects from systemic absorption [54]. [60], The only study to investigate intra-pleural bacterial products in MPM was negative, although risk of bias was high across all domains [47]. In contrast, an observational study reported longer survival in MPM patients who experienced pleural infection, compared with MPM patients without infection [61]. Although the results of this small study should be interpreted cautiously, they raise the possibility that bacteria in the pleural space may be associated with longer survival in MPM. Suitably-powered randomised trials are required to determine whether bacterial products could be therapeutically useful in MPM.

BCG was associated with a survival benefit in both studies that employed it, although they were non-randomised and at high risk of bias in several domains. This is in keeping with the established anti-neoplastic effect of intra-vesical BCG in bladder cancer. [28,62–66], BCG also exerts a cytotoxic effect in melanoma, inducing tumour regression and significant prolongation of survival following intra-lesional administration [67]. The exact mechanism of action is unknown, but is likely to involve activation of CD4 + and CD8 + T lymphocytes and release of cytokines, such as interferon-gamma and tumour necrosis factor [68–71]. On the basis of its activity in other tumour-types, BCG warrants further investigation and, since it appears to be effective as a topical treatment for localised tumours, MPM is a conceptual fit as a potential target disease.

4.2. Limitations of study

The main limitation of this study was the quality of data available for inclusion. The majority of studies were observational and therefore at high risk of selection bias, and many were also retrospective, with an associated risk of reporting bias.

MPE is a heterogeneous disease which includes a wide range of patients, disease processes and treatment options. Several different bacterial products have been used in clinical trials, at different doses and regimens. As discussed above, as well as preventing meta-analysis, this heterogeneity makes interpretation difficult, particularly in terms of evaluating the effect of individual bacterial products in specific disease processes.

Finally, five papers were not available for full text review, and a further six did not report the outcomes of interest despite mentioning them in the methodology. Attempts to contact the authors for raw data were unsuccessful, perhaps unsurprisingly given almost 3 decades had passed since publication in some cases. Missing papers and outcomes raise the possibility that the review could be affected by publication bias.

5. Conclusion

This systematic review of the survival effects of intra-pleural bacterial immunotherapy revealed a lack of good quality evidence, with high or unclear risk of bias in at least one domain in all included studies. Suitably powered, well-designed, prospective randomised trials are warranted to evaluate whether bacterial products could be of therapeutic benefit in pleural malignancy.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2018.06.002.

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